Commentary

From bacteria to mitochondria: Aconitase yields surprises

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More than a century of biochemical investigation has brought a thorough understanding of metabolism in a wide variety of organisms in the biosphere. Organisms inhabiting diverse niches ranging from anaerobic to highly aerobic environments share a surprisingly similar spectrum of enzyme activities, which are organized into pathways that provide the products for growth and development. Although this great body of information provides details of metabolic pathways, relatively little is understood regarding the forces that drove the evolution of metabolic pathways or the evolution of the specific enzymes involved. Nowhere is this more evident than in the evolution of mitochondria. Mitochondria participate in a wide range of activities in eukaryotic cells, from energy metabolism to apoptotic signaling in multicellular organisms. This is quite remarkable considering that mitochondria are believed to have evolved from a bacterial endosymbiont (1). Current views on the origins of mitochondria look to a α-proteobacterium endosymbiont as the ancestor (1). The α-proteobacteriaciae includes present-day intracellular parasites such as *Rickettsia prowazekii*. It was the completion of the *R. prowazekii* genome sequence in 1998 that revealed the close relationship of mitochondria to the α-proteobacteriaciae (2). In this issue of PNAS, Baughn and Malamy (3) report the identification and characterization of genes encoding the enzymes of the oxidative branch of the Krebs cycle in *Bacteroides flagilis*. Based on phylogenetic analyses, these authors suggest a common origin of the enzymes of the oxidative branch of the Krebs cycle in *Bacteroides* sp. and mitochondria. This surprising finding supports a polyphyletc origin of the mitochondrial Krebs cycle, and raises the question of what forces drove the evolution of the mitochondrial proteome.

Phylogenetic analyses of genes encoded in the mitochondrial genome, as well as nuclear-encoded genes for mitochondrial proteins, reveal that about half of the proteins in present-day mitochondria are most closely related to bacterial proteins with the same activity (1). A majority of the genes encoded in mitochondrial genomes can be traced back to the analogous genes in α-proteobacterial genomes (1, 4). However, only a small fraction of all of the proteins that make up the mitochondrial proteome can be traced back to an origin in the α-proteobacteria. The remaining mitochondrial proteins suggest an origin from other eubacteria or the primitive eukaryotic host. The serial endosymbiont hypothesis posits that present-day eukaryotes evolved through a series of endosymbiotic events between bacteria and a primitive eukaryote, ultimately giving rise to the complex organellar and metabolic structure seen today (5). Baughn and Malamy's report (3) adds to the notion that perhaps a consortium of bacterial endosymbionts contributed to the evolution of mitochondria. Until this study, all other bacterial aconitases, including the α-proteobacterial aconitase, fell into either the aconitase group similar to eukaryotic iron regulatory proteins/cytosolic aconitase (the AcnA/IRP group) or the aconitase family found only in bacteria, called the AcnB group (3). The report by Baughn and Malamy (3) describes the first bacterial aconitase to be closely related to the mitochondrial aconitase group. Their results suggest that metabolic pathways have evolved through selection of enzymes from diverse species.

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Bacteroides fragilis is a member of the Cytophaga-Flavobacterium-Bacteroides (CFB) phylum. Bacteria of the CFB phylum inhabit widely diverse environments ranging from anaerobic soils to the human gut. Members of this phylum also have been found in present-day endosymbiotic relationships (6). The significance of the oxidative branch of the Krebs cycle to the ability of CFB group bacteria to inhabit a particular niche is not clear. Baughn and Malamy propose that the oxidative branch enzymes provide an alternative, heme-independent pathway for synthesis of α-ketoglutarate, a crucial precursor for several biosynthetic pathways. Thus these enzymes may provide the organism with the flexibility to thrive under a wider variety of nutrient availability.

Aconitase is an interesting enzyme to consider in the evolution of metabolic pathways. Aconitases are Fe-S proteins, having a [4Fe-4S] cluster in the enzymatically active form and are considered to be ancient enzymes (7). The three phylogenetic categories of aconitase described above are equally effective as enzymes, and hence it is not obvious why a particular aconitase would have evolved to provide a particular metabolic function (8, 9). Where aconitases differ is in their relative sensitivity to oxygen-mediated inactivation. Although all aconitases are inactivated by exposure to oxygen, the AcnA/IRP group appears to be less sensitive to air inactivation compared with the other aconitases (9). It therefore might make sense that organisms that carry out aerobic respiration would use the more stable AcnA/IRP aconitase for its metabolism. However, *Escherichia coli* deviates from this pattern. *E. coli* has two aconitases, one of the AcnA/IRP family and the other a member of the AcnB family (10). AcnB is the major aconitase for logarithmic growth in aerobic *E. coli* cultures; its synthesis is repressed in stationary phase and by anaerobiosis (11). AcnA is induced in stationary phase or during oxidative stress (10, 11). Thus, in *E. coli* the more oxygen-sensitive aconitase serves as the primary aconitase during aerobic metabolism. A similar situation exists in the eukaryotic cell. Mitochondrial aconitase is the major aconitase for respiration and it is also more sensitive to oxygen than is the cytosolic IRP1 isoform. The cytosolic isoform can support some biosynthetic pathways and may be important for resistance to oxidative stress (12,

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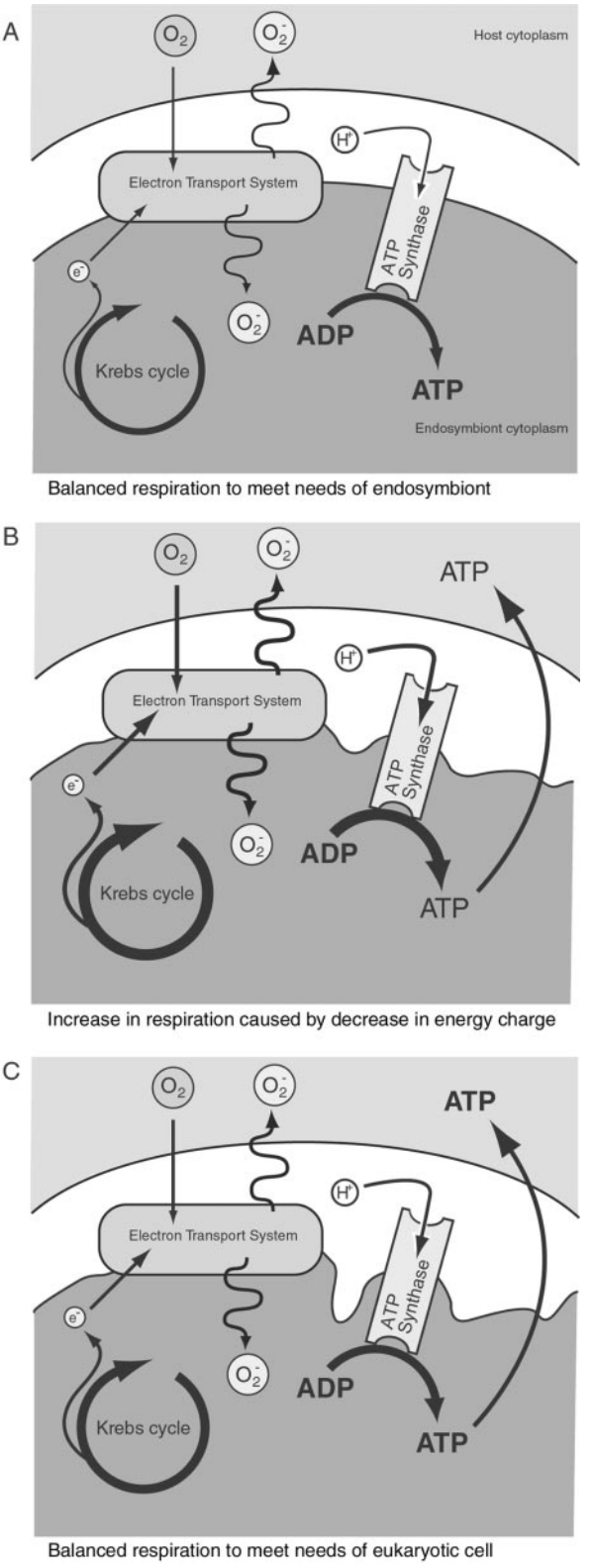

Fig. 1. Metabolic evolution of endosymbiont to organelle. (*A*) The early endosymbiotic association of a respiratory competent α-proteobacterium with a primitive eukaryote. The host and/or endosymbiont would have initially benefited from intermediates of each organism's unique balanced metabolism. (*B*) The acquisition of ATP exporting functions by the endosymbiont allows the host to use energy (in the form of ATP) produced by the endosymbiont, causing an energy debt (smaller ATP in endosymbiont cytoplasm). The organism responds by increasing respiration to generate more ATP (thicker lines for Krebs cycle and ATP synthesis). An increase in electron transport leads to higher superoxide (O_2^-) production. (*C*) Adaptation of mitochondrial aconitase to new organelle provides an efficient feedback regulation to the Krebs cycle, allowing the organelle to balance respiration and superoxide production.

13). It is possible that the higher sensitivity of mitochondrial aconitase and AcnB in *E. coli* provides for feedback regulation of aconitase activity during aerobic metabolism. The accumulation of superoxide from the electron transport chain would cause reduction in aconitase activity to slow down the Krebs cycle and reduce the flow of electrons into the electron transport chain. The greater sensitivity of mitochondrial aconitase and AcnB to reactive oxygen species may provide aerobic organisms with a crucial and sensitive feedback control for aerobic metabolism.

Recent evidence suggests that the *Bacteroides* aconitase is inactivated when cells or their extracts are exposed to oxygen (14). Because *Bacteroides* sp. are obligate anaerobes, the relative sensitivity of key enzymes to oxygen might seem irrelevant. However, many *Bacteroides* sp. are inhabitants of animal intestinal tracts and are opportunistic pathogens. Therefore, these organisms experience periodic exposure to oxygen, either in being transferred from one host to another or upon invasion of other regions of the body. The ability to survive these temporary aerobic conditions is crucial to this group of organisms. When *Bacteroides* sp. are exposed to an aerobic environment they stop growing. Pan and Imlay (14) reported recently that the failure of *Bacteroides* sp. to continue to grow when exposed to oxygen is because of oxygen-mediated inactivation of several Fe-S cluster-containing enzymes of metabolism. Recovery from air exposure depended on the ability of the air-exposed cells to repair the damage to these enzymes (14). Thus, for *Bacteroides* sp. it may be more important to be able to readily repair a damaged enzyme rather than prevent the damage.

Do the aconitases provide us with insight? The accumulating evidence suggests that the aconitases may differ in the way they are repaired in cells after oxidation-mediated Fe-S cluster disruption. Exposure of all aconitases to reactive oxygen converts the Fe-S cluster from a 4Fe cluster to a 3Fe cluster (9, 15–17). In the cell, removal of the oxidant appears to result in rapid conversion of the 3Fe cluster to a 4Fe cluster in the AcnB group of aconitases and most likely the mitochondrial enzyme (15, 18, 19). Recent evidence also suggest that such a 3Fe to 4Fe repair process occurs with the *Bacteroides* sp. aconitase (14). Although the AcnB and mitochondrial aconitases are very sensitive to oxygen-mediated inactivation, when oxygen is removed enzyme activity is rapidly recovered through iron-mediated repair of the Fe-S cluster. The repair of oxidant-damaged AcnA/IRP aconitases appears to be slower and most likely requires complete cluster disassembly (16). This characteristic of the AcnA/IRP ac-

onitases may be beneficial because of its dual role in gene regulation, which requires a pool of apo protein (20–22). However, it may make enzymatic response to changing conditions of oxidative stress much more sluggish. Thus, the aconitase of mitochondrial-like CFB group bacteria may enhance their survival during brief periods of air exposure (14).

So why did eukaryotes adapt the CFB aconitase for respiration in mitochondria versus the aconitase descended from the α-proteobacterium endosymbiont that gave rise to mitochondria? As pointed out by Baughn and Malamy (3), the aconitase of *R. prowazekii* is of the AcnA/IRP lineage and not like the mitochondrial aconitase. Thus, we are forced to consider what aspects of the mitochondrial aconitase made it better suited to its role in aerobic metabolism than the aconitase presumably encoded by the α-proteobacterial endosymbiont. If we accept that an ancestral α-proteobacterium gave rise to mitochondria, then it is likely that it already had an intact Krebs cycle. In fact, it is the aerobic respiratory function that is believed to have been the unique contribution of the α-proteobacterium to the symbiotic relationship. At the beginning of the symbiotic relationship each organism may have simply benefited by an exchange of metabolic intermediates and byproducts of each organism's unique metabolism (Fig. 1*A*). For example, modern-day rice weevils have an endosymbiotic relationship with *Sitophilus oryzae* principal endosymbiont (called SOPE), which supply the weevils with a variety of vitamins (23, 24). At some point in the evolution of mitochondria a pivotal event sealed the fate of the endosymbiont to become an organelle. Although we cannot know what this event was, a good candidate is the introduction of ATP export activities into the endosymbiont so that the host could use energy generated by the endosymbiont/organelle.

The export of ATP would have had profound consequences for the endosymbiont and the host as well. Initially, the endosymbiont would have experienced an energy debt as some of its energy capital was siphoned off by the host cell. It is expected that the endosymbiont would have increased respiration to compensate for this energy debt (Fig. 1*B*). However, a consequence of increased respiration would be generation of higher amounts of superoxide radical, leaking from the electron transport system (ETS). Superoxide generated by the ETS would inhibit sensitive Fe-S proteins in the endosymbiont and the host, including aconitase. So a burst in respiration to compensate for the energy debt would be followed by suppression of respiration caused by inhibition of key enzymes. After superoxide levels had returned to within normal levels respiration could speed up again and the cycle would repeat. Before adapting the CFB aconitase for function in the endosymbiont, the resident AcnA/IRP-like aconitase may have responded to cycles of high respiration and oxidative stress sluggishly, allowing the buildup of higher amounts of superoxide. It is also envisioned that recovery of activity from the aconitase of the endosymbiont would be slow because the superoxide damage had resulted in complete cluster removal. The adaptation of the CFB aconitase to function in the early mitochondrion would have provided an effective means for fine-tuning respiration and superoxide production to meet the metabolic needs of the cell in a safe way (Fig. 1*C*).

Did eukaryotes acquire the mitochondrial aconitase gene from CFB group bacteria or did the bacteria acquire the gene from the primitive eukaryotic host? Baughn and Malamy (3) suggest that a CFB group endosymbiont contributed the mitochondrial aconitase gene to the nucleus of the primitive eukaryote. Currently, there is not enough evidence to decide in which direction the gene transfer occurred. Either way the results presented in their article are strong evidence in support of a serial endosymbiont theory for the evolution of eukaryotes. In fact, one could envision a primitive eukaryote on the early aerobic earth with a consortium of endosymbionts that provide activities that both contribute to its metabolism and protect the cell from the deleterious effects of the early oxygen atmosphere. The study by Baughn and Malamy was aided by the completion of the genome sequence of *Bacteroides flagilis*. As more genome sequences are completed it is clear that new insights regarding the forces that shape organisms and the metabolic functions within will arise. Aconitase was one of the first enzymes for which dual, mutually exclusive functions were demonstrated to have biological importance (25). It is likely that this ancient and unique enzyme will continue to surprise us as we seek to better understand its role in cellular metabolism.

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